

Minimal increase in larval and adult performance of the biological control agent *Oxyops vitiosa* when fed *Melaleuca quinquenervia* leaves of different nitrogen levels

G.S. Wheeler*

USDA/ARS Invasive Plant Research Lab and University of Florida, 3205 College Ave., Ft. Lauderdale, FL 33314, USA

Received 24 October 2001; accepted 25 September 2002

Abstract

Leaves from the invasive tree *Melaleuca quinquenervia* containing a range of nitrogen levels were fed to neonate and adult *Oxyops vitiosa* to determine the influence of plant nutrition on insect survival, growth, development, and fecundity. Larvae fed the low-fertilizer leaves (30 g/11.4-liter pot; 15–9–12, N–P–K) had less than one half the survival ($30 \pm 5.0\%$) of larvae fed the high-fertilizer (90 g/pot) leaves ($65 \pm 10.4\%$). Additionally, the biomass of prepupae, pupae, and adults was each significantly greater (each 1.15-fold) in larvae fed leaves from the high-fertilizer level. However, fecundity was not influenced by these fertilizer treatments. The effect of five fertilizer treatments (30, 90, 135, 180, and 225 g/pot) applied to *M. quinquenervia* on *O. vitiosa* larval survival, growth, development, and feeding efficiency was determined in a second experiment. These results indicated that slight increases occurred in food consumption and biomass when larvae were fed leaves from the lowest and highest fertilizer treatments, respectively. Little if any evidence of compensatory feeding or increased food utilization efficiency was found on the low-nitrogen leaves. The results suggest that in mass rearing activities increased production of weevils will occur in high-fertilized (90 g/pot) treatments due to increased larval survival. However, adult fecundity will not be affected by fertilizer treatment. Several qualities of this species contribute to its success as a biological control, namely the adults are long-lived, which enables individuals to bridge periods when suitable leaves are available, and both larval and adult performance are apparently little affected by a wide range of nitrogen levels. Published by Elsevier Science (USA).

1. Introduction

The need for biological control of the Australian melaleuca tree *Melaleuca quinquenervia* (Cav.) Blake (Myrtaceae) in south Florida resulted in the 1997 release of *Oxyops vitiosa* Pascoe (Coleoptera: Curculionidae) (Center et al., 2000). Both the larvae and adults are specialist flush-leaf-feeders of this species (Purcell and Balciunas, 1994). In Australia, this insect is most abundant in situations where trees are growing rapidly, often from suckers or landscaped plants (Purcell and Balciunas, 1994). In south Florida, the greatest insect densities have developed where abundant growing tips of *M. quinquenervia* are maintained by frequent mowing (Center et al., 2000). Previous research highlighted the

importance of *M. quinquenervia* leaf quality for the survival, growth, and development of *O. vitiosa* larvae (Wheeler, 2001). For example, nearly all the *O. vitiosa* larvae died when fed leaves from branches that lacked emerging bud leaves. Associated with this high mortality were high leaf toughness values that exceeded 750 g/mm^2 compared with acceptable leaves with values between 200 and 350 g/mm^2 (Wheeler, 2001). However, when *O. vitiosa* larvae were fed flush leaves from branches with emerging leaves (bud stage 4, Van et al., USDA/ARS, Ft. Lauderdale, FL unpublished data), greater larval survival, reduced development time, and greater biomass were associated with relatively high foliar nitrogen and water levels (Wheeler, 2001). Although other inorganic nutrients are important for healthy plant growth, the nitrogen content of leaves is a widely accepted predictor of insect herbivore performance (Mattson, 1980; McNeill and Southwood, 1978; Slansky and Feeny, 1977; Strong et al., 1984). Additionally, the water

* Fax: 1-954-476-9169.

E-mail address: wheeler@ars.usda.gov.

content of leaves may be an important factor influencing herbivore performance, especially for tree-feeding herbivores like *O. vitiosa* (Scriber, 1977; Mattson and Scriber, 1987). This suggests that *O. vitiosa* larval performance (survival, growth, and development) and adult fecundity are influenced by young, high-quality foliage, and therefore, establishment, mass rearing, and redistribution of this species in Florida for biological control of *M. quinquenervia* will benefit from knowledge of these nutritional limitations.

Herbivore responses to variable food quality involve flexible behavioral and physiological processes that may ameliorate the negative effect of an unsuitable host. These compensatory responses typically fall into three different categories, including increased rates of food intake, consumption of additional tissues that complement the limiting nutrient, and increased digestive efficiency of the limiting nutrients (Simpson and Simpson, 1990; Slansky, 1993). Compensatory feeding for nutritionally suboptimal food may be a common adaptive response found in many herbivorous species. For example, food intake may increase more than 2-fold and food digestion may increase 1.3-fold in caterpillars fed diluted artificial diets (Wheeler and Slansky, 1991). The benefits of such a response may include increased herbivore biomass, shorter development time, and increased fecundity compared with individuals that lack this response (Slansky, 1993). However, one of the costs of such a response may include increased intake of potentially toxic materials contained in the leaves (Slansky and Wheeler, 1992). The objective of this study was to determine the impact of *O. vitiosa* larval and adult nutrition when fed leaves from *M. quinquenervia* grown at different fertilizer levels on pre-oviposition period, fecundity, and adult longevity. Moreover, the effect of different *M. quinquenervia* fertilizer levels on larval survival, growth, development, consumption, and digestive efficiency was determined.

2. Methods and materials

2.1. Plant quality

Plants of *M. quinquenervia* were germinated from seeds obtained from field sites in south Florida. These plants were grown in 1-liter pots connected to a drip irrigation system in a screenhouse located at the USDA/ARS, Invasive Plant Research Laboratory in Ft. Lauderdale, FL. Seedlings for the *Fertilizer Effect on Fecundity* study (see below) were transplanted during September 1998 and grown in one of two fertilizer levels 30 or 90 g/11.4-liter pot (Osmocote Plus 15–9–12, N–P–K; Scotts-Sierra Horticultural Products, Marysville, OH) in a slow-release ‘southern’ formulation. Seedlings for the *Fertilizer Effect on Larval Performance* study (see below) were

transplanted during September 1998 to one of five fertilizer levels, 30, 90, 135, 180, and 225 g/pot. All plants were grown in tanks that received rainwater and irrigation 3 times/week for approximately 3 months. Preliminary studies indicated that plants grown at the 30 g/pot level appeared similar to field plants grown under nitrogen-limited conditions as most of their leaves were yellow to light green and few emerging bud leaves were present. Plants grown at the 90 g/pot level appeared similar to field plants grown under conditions where nitrogen was not limited as the leaves were generally dark green and there were many growing tips present (Wheeler, 2001). The 90 g/pot level was the fertilizer treatment used for routine *M. quinquenervia*/*O. vitiosa* rearing and for a variety of experiments with these species. Plants were grown at the higher fertilizer levels (135, 180, and 225 g/pot) to determine if the larvae benefit from feeding on higher quality leaves for mass rearing of this biological control agent.

Branch tips ($n = 9$), including emerging bud leaves, were removed and used either for plant quality analysis or fed to larvae and adults as needed. For the *Fertilizer Effect on Fecundity* study (see below), leaves from positions 0 to 8 (counting from the tip toward the branch base) were collected, dried, and analyzed for nitrogen content in bulk from each fertilizer treatment. Nitrogen analysis of leaf samples was performed as described previously (Wheeler, 2001). To determine if differences in leaf nitrogen content occurred, an ANOVA was conducted comparing the percentage nitrogen in the bulked leaves from each treatment. For the *Fertilizer Effect on Larval Performance* study (see below), percent dry mass of individual leaves was determined gravimetrically ($n = 45$) by comparing the mass of leaves weighed fresh and after drying at 60°C for 48 h. Additionally, the nitrogen content of leaf pairs ($n = 3$) was determined by combining leaves 0 and 1, 2 and 3, 4 and 5, and 6 and 7. Percentages of nitrogen on both dry-mass and fresh-mass (percent dry mass nitrogen of leaves \times proportion of dry mass of leaves) bases were determined. To determine if the fertilizer treatments and leaf positions influenced leaf percent dry mass or nitrogen levels, the linear regression coefficients of these two effects were compared with analysis of covariance (ANCOVA; PROC GLM; SAS Institute, 1990) where leaf position served as the covariate. If the ANCOVA indicated that leaf position was not significant, the effect of fertilizer treatment was analyzed by ANOVA and the means were compared with a Ryan's Q mean comparison test ($P = 0.05$).

2.2. Fertilizer effect on fecundity

2.2.1. Adult performance and fecundity

Eggs were collected from our laboratory colony and 40 neonates were placed on flush leaves of either the 30

or the 90 g/pot treatments. Foliage and larvae were kept in petri dishes (15 × 2 cm) lined with moistened filter paper and sealed with parafilm to retain moisture. All insects were reared at 25°C, 90% RH, and L12:D12 h photophase. Prepupae were transferred to individual 30-ml plastic cups for pupation containing ground floral blocks (Smithers-Oasis, Kent, OH) (Wheeler and Zahniser, 2001). At emergence, adult male and female pairs ($n = 20$) were transferred to Plexiglas cylindrical cages (30 × 15 cm) and supplied with fresh *M. quinquenervia* tips. The eggs were counted and removed every three days. Adults were fed either flush leaves of the same fertilizer treatments upon which they had fed as larvae (treatments coded: 3030 or 9090) or flush leaves of the alternate fertilizer treatment (treatments coded: 3090 or 9030). Thus, we could distinguish between larval and adult nutrition on adult survival and performance. Moreover, we could determine the ability of adults to minimize the effects of a poor larval diet. Data were collected on larval survival, development time and biomass at the prepupal, pupal, and adult stages, the pre-oviposition period, adult longevity, and the total number and rate of eggs produced. To determine if significant differences occurred between the larval and adult diets, the insect performance data were analyzed by a two-way ANOVA and means were compared with a Ryan's Q mean comparison test ($P = 0.05$).

2.3. Fertilizer effect on larval performance

2.3.1. Larval performance

Eggs were collected from our laboratory colony and the neonates ($n = 15$) were placed on flush leaves of one of the fertilizer treatments (i.e., 30, 90, 135, 180, or 225 g/pot). All rearing conditions were as described above. The frass was removed, dried, and weighed. The leaves were replaced at least every three days or sooner if the larvae consumed 75% or more of the leaf fresh mass. Larval consumption was estimated gravimetrically as described previously (Wheeler and Halpern, 1999). Larvae were reared to the adult stage and data were collected on larval survival, larval consumption, development time to the prepupal, pupal, and adult stages, and prepupal, pupal, and adult biomass. To determine the influence of leaf nitrogen levels and insect sex on these nutritional parameters two-way ANOVAs were conducted followed by a Ryan's Q mean comparison test ($P = 0.05$).

2.3.2. Food digestion and conversion

Food digestion and conversion efficiencies were examined by ANCOVA where the effects of fertilizer treatment and insect sex were determined. Frass output was analyzed as the response variable and consumption served as a covariate providing information on food digestion and absorption similar to the ratio-based nu-

tritional index approximate digestibility (AD). Additionally, pupal biomass was analyzed as the response variable and food absorption served as a covariate providing information about the conversion of absorbed food to biomass similar to the ratio-based nutritional index efficiency of conversion of digested food (ECD) (Raubenheimer and Simpson, 1992; Wheeler and Halpern, 1999; Wheeler et al., 2001). When significant ANCOVA results were found, covariate-adjusted means were compared with a least square mean test while maintaining the experiment wise error rate at $P = 0.05$ with the Dunn-Šikák method (Sokal and Rohlf, 1981).

3. Results and discussion

3.1. Fertilizer effect on fecundity

3.1.1. Plant quality

The percent nitrogen of bulked leaves was influenced by the fertilizer treatments (Fig. 1). Leaves from the plants grown at a fertilizer level of 90 g/pot had ($F_{1,13} = 19.15$; $P = 0.0005$) greater percent nitrogen (mean \pm SE) ($1.71 \pm 0.01\%$ dry mass) than those grown at 30 g/pot fertilizer ($1.49 \pm 0.05\%$ dry mass).

Larval survival was greater ($F_{1,4} = 9.19$; $P = 0.0387$) when fed the high-fertilizer leaves ($65.0 \pm 10.4\%$) than those fed leaves from the low-fertilizer treatment ($30.0 \pm 5.0\%$; Fig. 2A). Biomass at the prepupal, pupal, and adult stages was influenced by both fertilizer treatments (prepupa: $F_{1,86} = 16.14$, $P < 0.0001$; pupa: $F_{1,87} = 15.85$, $P < 0.0001$; adult: $F_{1,89} = 12.51$, $P = 0.0006$) and sex (prepupa: $F_{1,86} = 32.35$, $P < 0.0001$; pupa: $F_{1,89} = 20.32$, $P < 0.0001$; adult: $F_{1,86} = 25.65$, $P < 0.0001$);

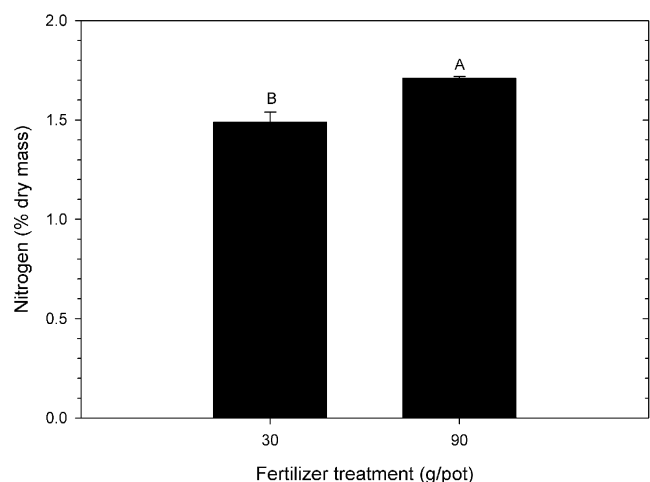


Fig. 1. Mean (\pm SE) percent nitrogen (dry mass) of leaves from *M. quinquenervia* plants fertilized at two levels. The fertilizer levels (Osmocote Plus 15–9–12, N–P–K; Scotts-Sierra Horticultural Products) were applied to 11.4-liter pots. Bars with the same letter were not significantly different ($P < 0.05$).

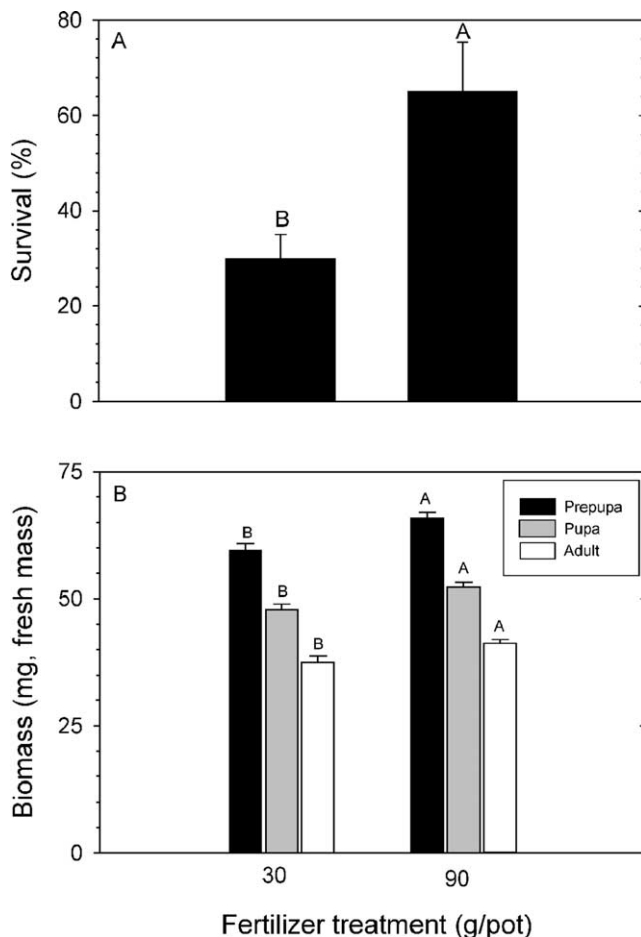


Fig. 2. Mean (\pm SE) *O. vitiosa* larval survival (A) and biomass (B) when larvae were fed leaves from *M. quinquenervia* plants fertilized at two levels. The fertilizer levels (Osmocote Plus 15–9–12, N–P–K; Scotts-Sierra Horticultural Products) were applied to 11.4-liter pots. Bars with the same letter, within a life stage (B), were not significantly different ($P < 0.05$).

however, the interaction between fertilizer treatment and sex was not significant for the biomass of these life stages (prepupa: $P > 0.07$; pupa: $P > 0.11$; adult: $P > 0.8$). In all developmental stages, greater biomass was achieved by larvae fed leaves from the higher fertilizer treatment (Fig. 2B). Similarly, females achieved about 1.15-fold greater biomass in all developmental stages than males (Fig. 3). Development times to the prepupal (14.7 ± 0.2 days), pupal (22.2 ± 0.3 days), and adult (30.5 ± 0.4 days) stages were not significantly influenced by either fertilizer treatment or insect sex.

3.1.2. Adult performance and fecundity

None of the adult performance and fecundity indices measured were influenced by the fertilizer treatments suggesting that adult feeding ameliorated the negative effect of low-nutrient leaves fed to larvae of the 3030 (30 g larval diet, 30 g adult diet) and 3090 (30 g larval diet, 90 g adult diet) treatments. Female pre-oviposi-

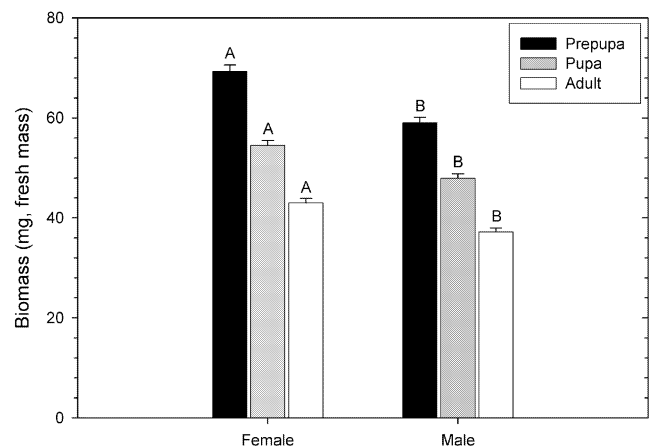


Fig. 3. Mean (\pm SE) *O. vitiosa* biomass to the prepupal, pupal, and adult stages by female and male individuals. The fertilizer levels (Osmocote Plus 15–9–12, N–P–K; Scotts-Sierra Horticultural Products) were applied to 11.4-liter pots. Bars with the same letter within a life stage were not significantly different ($P < 0.05$).

tional period averaged $47.4 (\pm 7.3)$ days) and was not significantly influenced by either larval or adult diets ($P > 0.8$). The longevity of the adult females averaged $256.6 (\pm 18.2)$ days and was not influenced by fertilizer treatment ($P > 0.5$). The experiment was terminated after 375 days and at that time 11 females of the original 40 were still alive and several continued to produce eggs. These included 1 female both from the 3030 diet (30 g larval diet, 30 g adult diet) and 3090 diet (30 g larval diet, 90 g adult diet), 5 females fed the 9030 diet (90 g larval diet, 30 g adult diet), and 4 females fed the 9090 (90 g larval diet, 90 g adult diet). The total number of eggs produced per female, including those still alive at the end of the experiment, averaged $283.0 (\pm 48.3)$ eggs; range 1–1069 eggs) and was not influenced by larval or adult diet ($P > 0.5$) and an average of $0.9 (\pm 0.2)$ eggs) was produced per female per day and was not influenced by either larval or adult diet ($P > 0.3$).

3.2. Fertilizer effect on larval performance

3.2.1. Plant quality

Percent dry mass of individual leaves was influenced by both fertilizer treatment and leaf position. The linear regression coefficients for the percent dry mass of leaves, as a function of leaf position from the tip toward the base, were determined for each fertilizer treatment. The slope coefficients for plants grown at only the two highest fertilizer treatments (180 and 225 g/pot) were significant indicating that percent dry mass decreased with leaf position away from tip. However, neither their slopes nor elevation coefficients (180 g/pot: $y = 27.5 (\pm 0.32) - 0.63 (\pm 0.07)x$; $r^2 = 0.93$; $P < 0.0001$) (225 g/pot: $y = 28.0 (\pm 0.60) - 0.43 (\pm 0.13)x$; $r^2 = 0.63$; $P < 0.0108$) differed significantly from one another (elevation: $P > 0.7$; slope: $P > 0.5$).

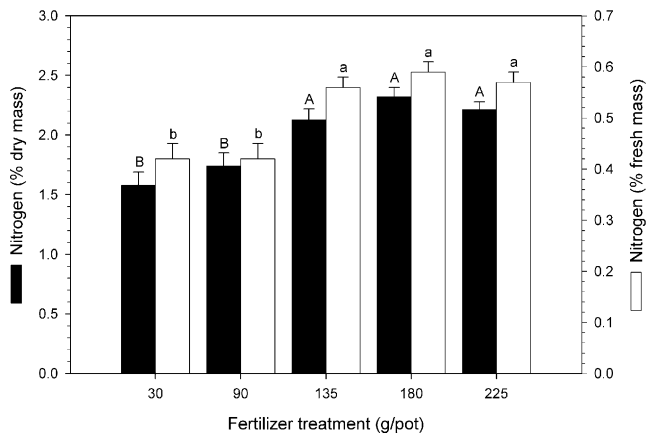


Fig. 4. Mean (\pm SE) nitrogen (dry mass and fresh mass) of leaves from *M. quinquenervia* plants fertilized at five levels. The fertilizer levels (Osmocote Plus 15–9–12, N–P–K; Scotts-Sierra Horticultural Products) were applied to 11.4-liter pots. Solid bars with the same upper-case letters (dry mass) or open bars with the same lower-case letter (fresh mass) were not significantly different ($P < 0.05$).

The percent leaf nitrogen increased in both dry mass and fresh mass (dry mass: $F_{4,54} = 11.56$; $P < 0.0001$; fresh mass: $F_{4,54} = 12.60$; $P < 0.0001$) at the higher fertilizer rates but was not influenced by leaf position (Fig. 4). The leaves from plants fertilized at rates equal to or greater than 135 g/pot had greater percent nitrogen than the leaves fertilized at lower levels (i.e., 30 and 90 g/pot).

3.2.2. Larval survival, growth, and development

Larval survival averaged $86.6 (\pm 4.7\%)$; range 73.3–100%) to the adult stage and was not significantly influenced by plant fertilizer treatment. Although the nitrogen levels of the 30 and 90 g/pot treatments in this study were similar to the corresponding treatments in the previous study (Fig. 1), survival of larvae fed the 30 g/pot leaves in this study was much higher (30 g/pot: $80.0 \pm 11.6\%$) than that of larvae fed the same treatment leaves in the previous study (see *Fertilizer Effect on Fecundity*; Fig. 2; 30 g/pot: $30.0 \pm 0.5\%$). Possibly seasonal changes or genetic differences in leaf chemistry other than nitrogen could account for these mortality differences; however, these factors were not measured here.

Consumption (dry mass) increased ($F_{4,62} = 3.45$; $P = 0.0131$) only for larvae fed the 30 g/pot leaves compared with those fed the 90 g/pot leaves (Fig. 5A). However, when assessed on a fresh-mass basis, possibly most relevant to larval nutrition (Slansky, 1993; Wheeler, 2001), no statistical change in consumption was detected in larvae fed leaves from the different treatments ($F_{4,62} = 2.16$; $P = 0.0845$; Fig. 5A). Pupal biomass was influenced by both fertilizer treatment ($F_{4,59} = 3.17$; $P = 0.0201$) and insect sex ($F_{1,59} = 20.19$; $P < 0.0001$). However, only female pupal biomass was influenced by fertilizer treatments as pupal biomass of females was greater when fed leaves from plants fertil-

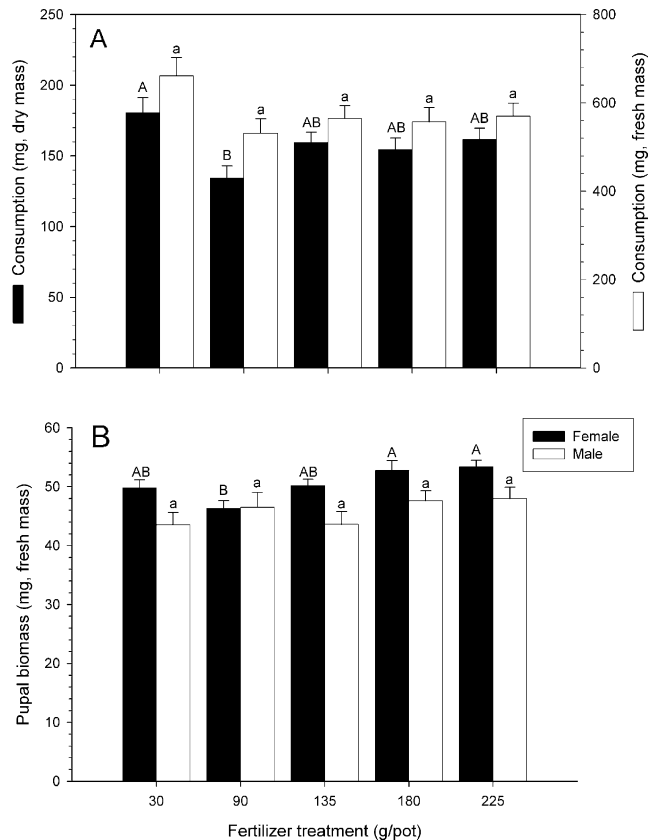


Fig. 5. Mean (\pm SE) *O. vitiosa* consumption (A; dry mass and fresh mass) and pupal mass (B; least square mean \pm SE) of larvae when the larvae were fed leaves from *M. quinquenervia* plants fertilized at five levels. The fertilizer levels (Osmocote Plus 15–9–12, N–P–K; Scotts-Sierra Horticultural Products) were applied to 11.4-liter pots. Pupal biomass values are covariate-adjusted for differences in food digestion. Solid bars with the same upper-case letters or open bars with the same lower-case letter were not significantly different ($P < 0.05$).

ized at the 180 and 225 g/pot treatments compared with females from the 90 g/pot fertilizer rate. Male pupal biomass was not influenced significantly by fertilizer treatments ($P > 0.3$; Fig. 5B). As in the previous study (*Fertilizer Effect on Fecundity*), female biomass was also greater than male biomass in the prepupal ($F_{1,59} = 15.5$; $P = 0.0002$), pupal ($F_{1,59} = 20.19$; $P < 0.0001$), and adult ($F_{1,59} = 24.04$; $P < 0.0001$) stages. However, none of the two-way interactions between fertilizer treatment and insect sex were significant. The nutritional parameters, development time to the prepupal (15.7 ± 0.2 days), pupal, (22.8 ± 0.3 days), and adult (30.6 ± 0.3 days) stages, were not statistically influenced by fertilizer treatments, insect sex, or their interaction.

3.2.3. Food digestion and conversion

Food digestion, as analyzed by ANCOVA, was not influenced statistically by fertilizer treatment, insect sex, or their interaction. However, the efficiency of digested food conversion to insect tissue was influenced by

fertilizer treatment ($F_{4,58} = 3.15$; $P = 0.0205$), and insect sex ($F_{1,58} = 20.23$; $P < 0.0001$), but not their interaction. The female covariate-adjusted pupal biomass for the 180 and 225 fertilizer treatments were greater than that of the 90 g/pot treatment (Fig. 5B). These results indicate that larvae fed leaves from the two highest fertilizer plants converted absorbed food more efficiently to insect biomass than those fed leaves from the 90 g/pot plants. No change in conversion efficiency was found in males. The average ratio-based nutritional indices suggest that 47.3 ($\pm 1.7\%$) of the ingested food was digested and absorbed (AD) and 66.9 ($\pm 1.1\%$) of the digested food was converted to insect biomass (ECD).

These results indicate that *O. vitiosa* larvae exert little, if any changes, in their performance parameters, consumption, and nutrient utilization in response to the nutritional content of leaves. Despite this apparent lack of compensation, little biomass difference occurred in larvae fed the low-nitrogen *M. quinquenervia* leaves. Although consumption (dry mass only) increased significantly only in larvae fed the 30 g/pot leaves compared with those fed the 90 g/pot leaves, no such response was found with the consumption of fresh mass. For this to be compensatory feeding, an increase in fresh-mass consumption, a more realistic indicator of compensatory feeding, should have also occurred (Slansky, 1993). Additionally, the efficiency of converting digested food to pupal biomass increased only in females fed the highest fertilizer treatments (180 and 225 g/pot). However, if this was a compensatory response the increase should have occurred when fed the low nitrogen leaves.

Such flexible feeding and nutrient utilization are common responses among insects and may mitigate the variable nutritional quality of their host (Simpson and Simpson, 1990; Slansky, 1993). These responses are widely regarded as adaptive as they lead to decreased exposure to natural enemies during the most vulnerable larval stage (Bernays, 1997; Loader and Damman, 1991). Furthermore, the nitrogen content of weevil food has been commonly associated with improved survival, developmental time, adult biomass (Hunt et al., 1993), relative growth rates (Wheeler and Center, 1997), and egg production (Cram, 1965a,b; Heard and Winterton, 2000; Hilliard and Keeley, 1984a,b; Maier, 1983; Room et al., 1989; Sands et al., 1983). Not only did our results indicate little if any compensation for low quality leaves but they also indicated little improvement in performance when fed higher quality leaves. A statistically significant, though slight, increase (1.15-fold) occurred in prepupal, pupal, and adult biomass when fed the 90 g/pot leaves compared with those fed the 30 g/pot leaves. Possibly the greatest impact of the fertilizer treatment on the insect's overall ability to reproduce and multiply was the increased mortality (nearly 2.2-fold) in larvae fed the 30 g/pot leaves compared with the 90 g/pot leaves (see *Fertilizer Effect on Fecundity*). However, this increased

larval mortality was not repeated in the second experiment (see *Fertilizer Effect on Larval Performance*) possibly because the nitrogen level of the 30 g/pot plants was slightly higher ($1.58 \pm 0.11\%$) than in the previous experiment ($1.49 \pm 0.05\%$). Thus, although the number of surviving females was influenced by fertilizer treatment, fecundity per female was not affected when either, or both, the larvae and the adults were fed leaves of different nitrogen content.

The apparent lack of compensatory responses seen here may be related to the potentially toxic terpenoids found in the leaves of *M. quinquenervia*. We have reported relatively high concentrations of several mono- and sesqui-terpenoids extracted from the leaves of *M. quinquenervia* (Wheeler et al., 2002a,b). These include α -pinene, 1,8-cineole, α -terpineol, β -caryophyllene, (*E*)-nerolidol, and viridiflorol, many of which are important mediators of insect-plant interactions (Gershenzon and Croteau, 1991; Langenheim, 1994). Possibly the larvae did not increase consumption when fed low-nitrogen leaves as they were unable to metabolize an increased dose of these terpenoids. The larvae may be feeding at a rate that allows detoxification of these terpenoids, converting them into innocuous and easily excreted forms (Brattsten, 1992). To exceed this rate may saturate the enzymes (e.g., polysubstrate monooxygenase) resulting in intoxication (Slansky, 1992; Slansky and Wheeler, 1992). Thus, because of the high concentration of potentially toxic terpenoids in the leaves of this plant, the *O. vitiosa* larvae may be unable to increase consumption to increase acquisition of limited nutritional resources.

Alternatively, the larvae may benefit little by decreasing development time, which may be associated with reduced exposure during the vulnerable larval stage to generalist predators. Other research indicates that in Florida, the larvae of this species sequester dietary terpenoids from the leaves of *M. quinquenervia* and deposit these compounds on their integument, often in greater concentrations than contained in the leaves (Wheeler et al., 2002a,b). Although the larvae are well protected from one of the most common invertebrate generalist predators in Florida (Elvin et al., 1983; Kharboutli and Mack, 1991), more rapid larval development may benefit this species in their native Australia where they are frequently attacked by a specialist tachinid parasitoid (Purcell and Balcunas, 1994). The significance of these defenses against generalist versus specialist natural enemies in the native range of this insect could resolve this apparent contradiction.

The metabolic cost of sequestering defensive compounds is difficult to assess because of the many factors involved in the process (Rowell-Rahier and Pasteels, 1992). This metabolic cost may be very low with little impact on insect biomass when consuming increased concentrations of allelochemicals (e.g., Bowers, 1988). Alternatively, the cost of sequestration may be relatively

high as demonstrated in neonate *Danaus plexippus* L. (Lepidoptera: Danaidae), where mortality increased when individuals consumed higher concentrations of cardenolides in plants (e.g., Zalucki et al., 1990). However, an additional cost of sequestration of plant defensive compounds, as demonstrated in the present study, may be the limited ability to compensate for low-nutrient food by increasing consumption rates to ameliorate their negative impact.

In previous work conducted in Australia, the pre-oviposition period of this species averaged between 42 and 46 days. Additionally, the females, when laying eggs, produced on average 1.2–1.8 eggs per day, 470 eggs per female, and at least some individuals continued laying for more than 200 days (Purcell and Balciunas, 1994). The results presented here generally support these findings; however, this study reports lower values for egg production both per female (283.0 eggs/female) and per female per day (0.9 eggs/female/day). Moreover, the results presented here indicate that adult longevity (>375 days) may exceed the previous estimates. Female weevils may live and continue laying eggs for over one year. This prolonged adult stage undoubtedly allows individuals to bridge periods when seasonal flush growth is unavailable.

The results of this study indicate that, despite a 7.5-fold increase in fertilizer levels applied to *M. quinquenervia* plants, there was only a 1.4-fold increase in foliar nitrogen levels. Although significant increases occurred in the levels of nitrogen in the leaves, no increase was evident when more than 135 g/pot was applied. Moreover, the nitrogen level of these plants fertilized with higher nitrogen levels never exceeded 2.5% dry mass. These relatively low nitrogen levels are consistent with a previous field study that reported 0.8–2.3% (dry mass) nitrogen in *M. quinquenervia* leaves collected from several sites in Florida (Wheeler, 2001). Similarly, these low foliar nitrogen levels were also reported from several *Eucalyptus* species in Australia (Fox and Macauley, 1977). The herbivore species is apparently well adapted to the relatively low nitrogen levels of its host, although the mechanism does not appear to include a compensatory response. In terms of mass production of weevils for biological control, little or no benefit would result in agent production when growing *M. quinquenervia* plants in excess of 135 g/pot fertilizer.

The implications of these findings for biological control of *M. quinquenervia* are several. First, the mass production of weevils at high fertilizer levels will improve larval survival. However, adult rearing can occur even on low-fertilizer plants with little effect on longevity or fecundity. Second, when considering environmental factors for the selection of agent release sites, nascent populations of agents will establish and build up populations more rapidly in high-fertilizer sites because of this increased larval survival. If biological control agents

are reared in nursery sites for local production and redistribution, fertilization of the plants will benefit larval survival and in this way the ultimate production of the colony.

Acknowledgments

I am indebted to the technical assistance of Luke Kasarjian, Rosa Leidi-Ferrer, University of Florida, Mark Endries, AmeriCorps, Student Conservation Association and Drs. P.D. Pratt and T.D. Center, USDA/ARS, Ft Lauderdale, FL for valuable comments on a previous draft of the manuscript. Financial support was provided by Florida Department of Environmental Protection and Dade County Department of Environmental Resource Management.

References

- Bernays, E.A., 1997. Feeding by lepidopteran larvae is dangerous. *Ecol. Entomol.* 22, 121–123.
- Bowers, M.D., 1988. Chemistry and coevolution: iridoid glycosides, plants, and herbivorous insects. In: Spencer, K.C. (Ed.), *Chemical Mediation of Coevolution*. Academic Press, New York, pp. 133–165.
- Brattsten, L.B., 1992. Metabolic defenses against plant allelochemicals. In: Rosenthal, G.A., Berenbaum, M.R. (Eds.), *Herbivores: Their Interactions with Secondary Plant Metabolites*, second ed.. Academic Press, New York, pp. 176–242.
- Center, T.D., Van, T.K., Rayachhetry, M., Buckingham, G.R., Dray, F.A., Wineriter, S.A., Purcell, M.F., Pratt, P.D., 2000. Field colonization of the melaleuca snout beetle (*Oxyops vitiosa*) in south Florida. *Biol. Control* 19, 112–123.
- Cram, W.T., 1965a. Fecundity of the black vine weevil on strawberry with nitrogen supplied in the ammonium or nitrate form. *Can. J. Plant Sci.* 45, 295–296.
- Cram, W.T., 1965b. Fecundity of the root weevils *Brachyrhinus sulcatus* and *Sciopithes obscurus* on strawberry at different conditions of host plant nutrition. *Can. J. Plant Sci.* 45, 219–225.
- Elvin, M.K., Stimac, J.L., Whitcomb, W.H., 1983. Estimating rates of arthropod predation on velvetbean caterpillar larvae in soybeans. *Florida Entomol.* 66, 319–330.
- Fox, L.R., Macauley, B.J., 1977. Insect grazing on *Eucalyptus* in response to variation in leaf tannins and nitrogen. *Oecologia* 29, 145–162.
- Gershenson, J., Croteau, R., 1991. Terpenoids. In: Rosenthal, G.A., Berenbaum, M. (Eds.), *Herbivores: Their Interactions with Secondary Plant Metabolites*. Vol I: The Chemical Participants, second ed. Academic Press, San Diego, CA, pp. 165–219.
- Heard, T.A., Winterton, S.L., 2000. Interactions between nutrient status and weevil herbivory in the biological control of water hyacinth. *J. Appl. Ecol.* 37, 117–127.
- Hilliard, R.A., Keeley, L.L., 1984a. The effects of dietary nitrogen on reproductive development in the female boll weevil, *Anthonomus grandis*. *Physiol. Entomol.* 9, 165–174.
- Hilliard, R.A., Keeley, L.L., 1984b. Interactions between dietary nitrogen and simulated autumn conditions on diet consumption and reproductive development in the boll weevil, *Anthonomus grandis*. *Physiol. Entomol.* 9, 417–423.
- Hunt, D.W.A., Lintreuer, G., Salom, S.M., Raffa, K.F., 1993. Performance and preference of *Hylobius radialis* Buchanan, and

- H. pales* (Herbst) (Coleoptera: Curculionidae) on various *Pinus* species. *Can. Entomol.* 12, 1003–1010.
- Kharboul, M.W., Mack, T.P., 1991. Relative and seasonal abundance of predaceous arthropods in Alabama peanut fields as indexed by pitfall traps. *J. Econ. Entomol.* 84, 1015–1023.
- Langenheim, J.H., 1994. Higher plant terpenoids: a phytocentric overview of their ecological roles. *J. Chem. Ecol.* 20, 1223–1280.
- Loader, C., Damman, H., 1991. Nitrogen content of food plants and vulnerability of *Pieris rapae* to natural enemies. *Ecology* 72, 1586–1590.
- Maier, C.T., 1983. Influence of host plants on the reproductive success of the parthenogenetic two-banded Japanese weevil, *Callirhopalus bifasciatus* (Roelofs) (Coleoptera: Curculionidae). *Environ. Entomol.* 12, 1197–1403.
- Mattson, W.J., 1980. Herbivory in relation to plant nitrogen content. *Annu. Rev. Ecol. Syst.* 11, 119–161.
- Mattson, W.J., Scriber, J.M., 1987. Nutritional ecology of insect folivores of woody plants: nitrogen, water, fiber, and mineral considerations. In: Slansky Jr., F., Rodriguez, J.G. (Eds.), *Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates*. Wiley, New York, pp. 105–146.
- McNeill, S., Southwood, T.R.E., 1978. The role of nitrogen in the development of insect/plant relationships. In: Harborne, J.B. (Ed.), *Biochemical Aspects of Plant and Animal Coevolution*. Academic Press, London, pp. 77–98.
- Purcell, M.F., Balcunas, J.K., 1994. Life history and distribution of the Australian weevil *Oxyops vitiosa* (Coleoptera: Curculionidae), a potential biological control agent for *Melaleuca quinquenervia* (Myrtaceae). *Ann. Entomol. Soc. Am.* 87, 867–873.
- Raubenheimer, D., Simpson, S.J., 1992. Analysis of covariance: an alternative to nutritional indices. *Ent. Exp. Appl.* 62, 221–231.
- Room, P.M., Julien, M.H., Forno, I.W., 1989. Vigorous plants suffer most from herbivores: latitude, nitrogen and biological control of the weed *Salvinia molesta*. *Oikos* 54, 92–100.
- Rowell-Rahier, M., Pasteels, J.M., 1992. Third trophic level influences of plant allelochemicals, second ed.. In: Rosenthal, G.A., Berenbaum, M.R. (Eds.), *Herbivores: Their Interactions with Secondary Plant Metabolites*, vol. 2. Academic Press, San Diego, pp. 243–277.
- Sands, D.P.A., Scholtz, M., Bourne, A.S., 1983. The feeding characteristics and development of larvae of a *Salvinia* weevil *Cyrtobagous* sp. *Ent. Exp. Appl.* 34, 291–296.
- SAS Institute, 1990. *SAS/STAT User's Guide Version 6*. SAS Institute, Cary, NC.
- Scriber, J.M., 1977. Limiting effects of low leaf-water content on the nitrogen utilization, energy budget, and larval growth of *Hyalophora cecropia* (Lepidoptera: Saturniidae). *Oecologia* 28, 269–287.
- Simpson, S.J., Simpson, C.L., 1990. The mechanisms of nutritional compensation by phytophagous insects. In: Bernays, E.A. (Ed.), *Insect-Plant Interactions*, vol. II. CRC Press, Boca Raton, pp. 111–160.
- Slansky Jr., F., 1992. Allelochemical–nutrient interactions in herbivore nutritional ecology, second ed.. In: Rosenthal, G.A., Berenbaum, M.R. (Eds.), *Herbivores, Their Interactions with Secondary Plant Metabolites*, vol. 2. Academic Press, San Diego, pp. 135–174.
- Slansky Jr., F., 1993. Nutritional ecology: the fundamental quest for nutrients. In: Stamp, N.E., Casey, T.M. (Eds.), *Caterpillars: Ecological and Evolutionary Constraints on Foraging*. Chapman & Hall, New York, pp. 29–91.
- Slansky Jr., F., Feeny, P.P., 1977. Stabilization of the rate of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated food plants. *Ecol. Monogr.* 47, 209–228.
- Slansky Jr., F., Wheeler, G.S., 1992. Caterpillars' compensatory feeding response to diluted nutrients leads to toxic allelochemical dose. *Ent. Exp. Appl.* 65, 171–186.
- Sokal, R.R., Rohlf, F.J., 1981. *Biometry: The Principles and Practice of Statistics in Biological Research*, second ed. Freeman, New York.
- Strong, D.R., Lawton, J.H., Southwood, T.R.E., 1984. *Insects on Plants*. Harvard University Press, Cambridge, MA.
- Wheeler, G.S., 2001. Host plant quality factors that influence the growth and development of *Oxyops vitiosa*, a biological control agent of *Melaleuca quinquenervia*. *Biol. Control* 22, 256–264.
- Wheeler, G.S., Center, T.D., 1997. Growth and development of the biological control agent *Bagous hydrillae* as influenced by *Hydrilla* (*Hydrilla verticillata*) stem quality. *Biol. Control* 8, 52–57.
- Wheeler, G.S., Halpern, M.D., 1999. Compensatory responses of *Samea multiplicalis* larvae in response to different fertilization levels of the aquatic weed *Pistia stratiotes*. *Ent. Exp. Appl.* 91, 205–216.
- Wheeler, G.S., Slansky Jr., F., 1991. Compensatory responses of the fall armyworm (*Spodoptera frugiperda*) when fed water- and cellulose-diluted diets. *Physiol. Entomol.* 16, 361–374.
- Wheeler, G.S., Zahniser, J., 2001. Artificial diet and rearing methods for the *Melaleuca quinquenervia* (Myrtales: Myrtaceae) biological control agent *Oxyops vitiosa* (Coleoptera: Curculionidae). *Florida Entomol.* 84, 439–441.
- Wheeler, G.S., Slansky Jr., F., Yu, S.J., 2001. Food consumption, utilization and detoxification enzyme activity of larvae of three polyphagous noctuid moth species when fed the botanical insecticide rotenone. *Ent. Exp. Appl.* 98, 225–239.
- Wheeler, G.S., Massey, L.M., Southwell, I.A., 2002a. Anti-predator defense of the biological control agent *Oxyops vitiosa* is mediated by plant volatiles sequestered from their host plant *Melaleuca quinquenervia*. *J. Chem. Ecol.* 28, 297–315.
- Wheeler, G.S., Massey, L.M., Southwell, I.A., 2002b. Dietary influences on the terpenoids sequestered by the biological control agent *Oxyops vitiosa*: the effect of plant volatiles from different *Melaleuca quinquenervia* chemotypes and laboratory host species. *J. Chem. Ecol.* (in press).
- Zalucki, M.P., Brower, L.P., Malcolm, S.B., 1990. Oviposition by *Danaus plexippus* in relation to cardenolide content of three *Asclepias* species in the southeastern USA. *Ecol. Entomol.* 15, 231–240.